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Histomorphological analysis of the effect of rigid fixation on growing sutures in the rabbit

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SUMMARY. **The effect of internal rigid fixation (IRF) on bone growth was studied in an experimental model set up in the rabbit.**

The frontonasal suture of the right side was surgically bridged by a microplate. As reference for bone growth, four screws were placed symmetrically in the four bony segments including the frontonasal suture on both sides.

The suture development was followed dynamically for 40 days on the basis of the position of the screws established radiographically. The rate of bone formation along the sutural bones was evaluated by means of the tetracycline labelling technique. The structure of the newly formed bone and its degree of mineralization were respectively analysed under polarized light and with microradiography.

It was demonstrated that IRF prevents growth of the sutural membrane but not of the osteogenic process; as a consequence the constrained sutures soon undergo synostosis. This fact must be taken into consideration when IRF is employed in children in order to avoid delayed removal of the plate irreversibly stopping the growth of the constrained suture.

INTRODUCTION

In the treatment of maxillo-facial fractures, malformations, deformities and neoplasms, two osteosynthesis methods can be used: wire suturing and internal rigid fixation (IRF) with screws. In the past 15 years, the former has been replaced progressively by the latter technique since this offers the advantage of primary bone healing; additionally it maintains the osteotomy gaps and avoids the blocking of the intermaxillary area after surgery (*Curioni* and *Clauser*, 1991). The theoretical basis of the IRF technique is to restore the continuity of the fractured, traumatized or operatively caused bone edges by compression (*Spiessl*, 1976).

Several experimental models have been used to study the various stages of craniofacial growth (*Babler* et al., 1982; *Persing* et al., 1991a, b; *Sarnat* and *Wexler*, 1966), the regenerative capacity of different areas of the osteotomized cranial vault (*Alberius* et al., 1990), and to evaluate the validity of the different surgical techniques used for osteosynthesis (*Lin* et al., 1991; *Remmler* et al., 1992). However, most of these investigations have been performed using cephalometry (*Marshall* et al., 1991; *Miller* et al.. 1990; *Wong* et al., 1991) whereas histomorphological data are lacking. Such data are needed in determining the site of plate application and the timing of its removal.

For the above mentioned reasons, a study on the effects of IRF on bone growth was performed in an experimental model using rabbits.

MATERIALS AND METHODS

Eight male New Zealand white rabbits aged 4 weeks were used (average weight 550 g); the animals were kept on a free diet.

Under general anaesthesia (intravenous injection of 25 mg/ml sodium pentobarbital) the rabbits were prepared for surgery by shaving the region between the coronal suture and the tip of the nasal bone; this region was disinfected with a 3% tincture of iodine solution in alcohol. A 5 cm long sagittal frontonasal cut was made through the entire thickness of the integument. Once the periosteum was retracted, a Luhr microplate was secured (with two screws) across the right frontonasal suture. Two reference screws were symmetrically positioned on the left and right sides with respect to the frontonasal suture and the inter-nasal suture. The holes for the screws were prepared with a low speed micromotor, using physiological sterile solution as coolant. In two rabbits, used as controls, four reference screws were positioned in the same places as in the treated animals, but without the plate. A single dose of oxytetracycline (30 mg/Kg) was administered three times subcutaneously: (1) soon after the operation, (2) 30 days after the operation and (3) 24 h before sacrifice. All animals were sacrificed 40 days after the operation.

During the whole experimental period, the development of the sutures was followed-up dynamically

on the basis of the position of the reference screws evaluated by X-ray.

After removal of the soft tissues, the portion of the cranial vault containing the frontal and the nasal bones was fixed for 2 h (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2), buffer washed, alcohol dehydrated and embedded in methylmethacrylate. A series of sagittal sections (200 μm thick) of the cranial vault were obtained using a 1600 Leitz circular diamond microtome. The surface of the sections, perfectly polished with emery paper and alumina, was microradiographed at 5 KV and 2 mA using an italstructures device and low resolution Ilford EM film. The sections were subsequently ground to approximately 80 μm, polished on both sides with alumina and then microradiographed at 10 KV 8 mA on high resolution Kodak SO 343 film. Photographs were taken using a Zeiss photomicroscope III under ordinary, polarized and fluorescent light.

RESULTS

In control rabbits, the right and left frontonasal sutures have, on the whole, a V-shape, open posteriorly with the apex in front (Fig. 1). On X-ray, these sutures have a flame-like appearance and thus they do not display such well defined edges as the inter-frontal and inter-nasal sutures, which in man correspond to metopic and harmonic sutures respectively. Such flame-like appearance depends on the irregular bone margins of the sutures which, in turn, is due to an irregular degree of mineralization, and hence radiopacity.

Low resolution microradiography of the parasagittal sections through the nasal and frontal bones in control rabbits (Fig. 2) shows that the frontonasal suture has a double squamous architecture since the frontal bone (to the left in the photograph) has a process penetrating into a reciprocal indentation of the nasal bone. This frontal process becomes sharp as in man but it is bigger and extends laterally, resembling a blade.

The fluorescent labels, corresponding to the three administrations of oxytetracycline, are discontinuous, thus indicating that bone growth is not uniform along the margin of the suture. In fact, the rate of daily bone growth, presumed by measuring the distance between two successive tetracycline labels with an ocular micrometer, shows a high range of variation: from 5 to 20 μm/day. This uneven distribution of the fluorescent labels prevents consistent estimation of longitudinal bone growth. The newly formed bone, viewed under polarized light, appears to be made up of parallel-fibred- and woven-fibred-bone, having a high degree of mineralization, similar to the pre-existent bone, as shown by high resolution microradiography.

In treated rabbits, the unconstrained suture shows a structure and behaviour similar to those observed in control animals. On the other hand, the fixed suture does not grow significantly with time, as presumed from the distance between the two reference screws compared with the symmetrical ones in the unfixed side (Fig. 3).

Low resolution microradiography of sagittal sections demonstrates that, as opposed to the unconstrained side (Fig. 4), the sutural membrane is largely ossified and the bone edges fused underneath the plate (Fig. 5).

Contrary to the unconstrained suture side (Fig. 6), the rate of bone formation appears to be greatly reduced along the sutural bony edges underneath the plate; in fact only single tetracycline labels are present (Fig. 7). Correspondingly, the thickness (30 μm approx.) of the sutural membrane is significantly lower compared with the value (150 μm approx.) in the contralateral unconstrained side.

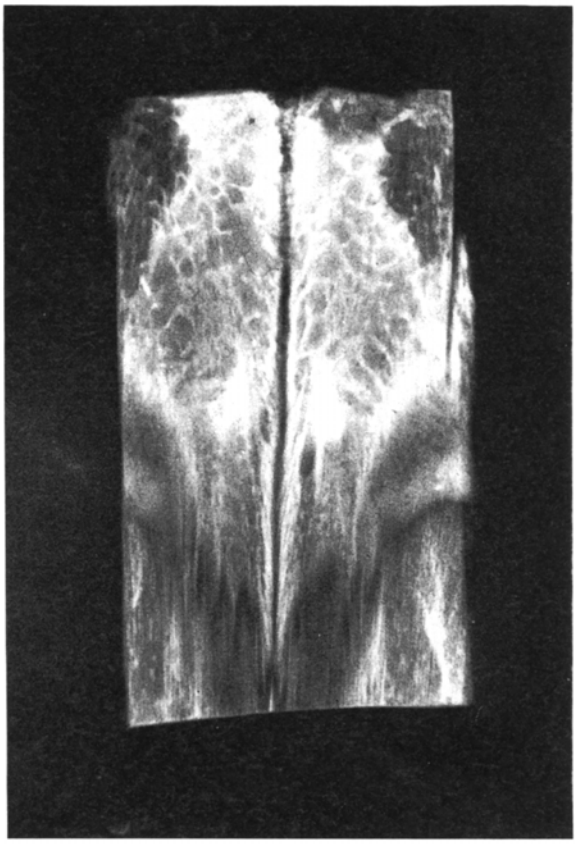

Fig. 1 – Anterior-posterior radiograph of the frontonasal suture in a 30-day-old rabbit.

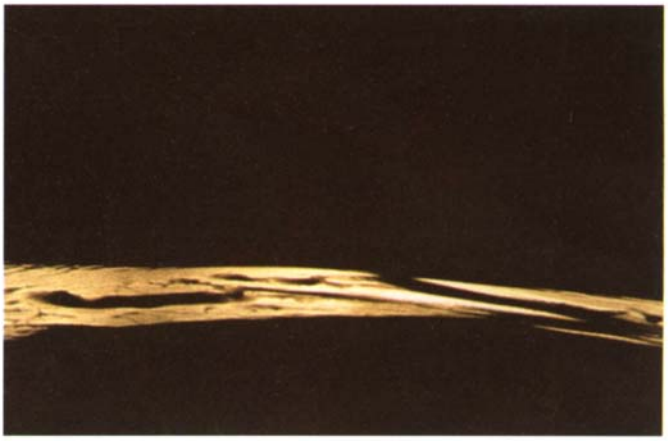

Fig. 2 – Microradiograph of a parasagittal section of the nasal and paranasal bones. Field width 10 mm.

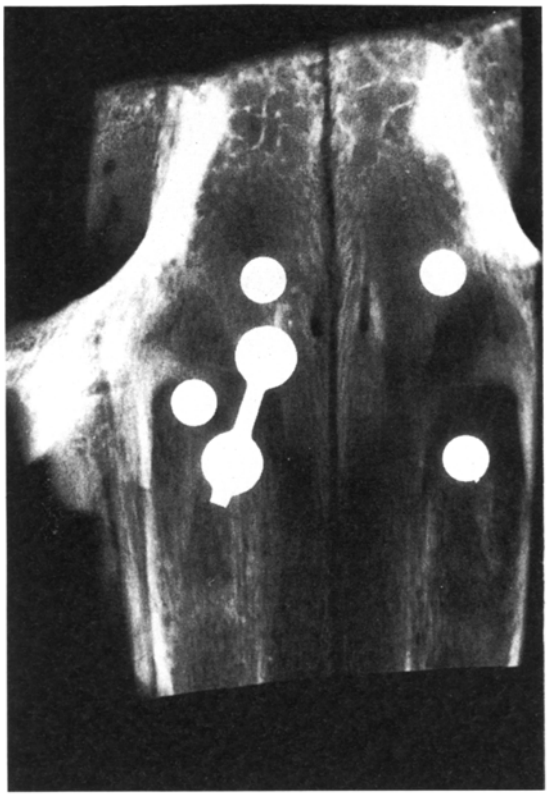

Fig. 3 – Radiograph showing the microplate and the four reference screws.

The newly formed bone underneath the plate is made up of parallel-fibred- and woven-fibred-bone as in control animals and in the unconstrained contralateral side; however, the new bone appears less mineralized than the pre-existing bone, as shown by the high-resolution microradiograph.

DISCUSSION

The New Zealand White rabbit was used since the linear dimensions of the growth pattern at its cranial base are similar to those in man and in primates. In fact, 50% of the growth of the anterior cranial base take place from the 1st to the 20th week after birth, while the posterior portion attains 70% of its growth by the end of the 1st week. Nevertheless, the angular measurements differ from those in primates (*Moore* and *Spence*, 1969), but this fact is irrelevant to the present study.

Surgery was performed on 30-day-old rabbits, i.e. animals which had been weaned since the incision would have made sucking impossible. Male rabbits were used to avoid hormonal interference with bone and suture growth. The Luhr microsystem (*Luhr*, 1988; *Luhr*, 1990) was used as the rigid fixation system since its size conforms to the cranial vault of the rabbit, and because it is made of vitallium, which has been shown to be a biologically inert alloy.

The frontonasal suture was chosen in our experiment because of its high rate of development, in fact it has been found to be 5 times higher than the frontoparietal suture (*Erickson* and *Ogilvie*, 1958), and to contribute in a predominant degree to the antero-posterior development of the skull during the first 6 months of postnatal life (*Hong* et al., 1968).

We attempted, as much as possible, to reproduce conditions similar to those occurring in children. It is worth noting, however, that our experimental model cannot be correlated to human craniofacial malformations due to early synostosis, since pathological sutures differ from sutures which are prevented from growing by physical fixation. It is noted, however, that sutures with malformations also usually stop growing as shown by the fact that Le Fort III osteotomy further reduces horizontal maxillary growth in patients with *Crouzon*, *Apert* and *Pfeiffer* syndromes (*Bachmayer* et al., 1986).

In 1955, *Selman* and *Sarnat* documented a remarkable development of the frontonasal suture during splanchnocranial growth; in 1957 the same authors, however, also showed that removal of the frontonasal suture did not modify the rabbit facial growth pattern. Thereafter, *Sarnat* and *Wexler* (1966) provided experimental proof that the development of the mid-facial skeleton depends on the integrity of the nasal septum cartilage. Finally, *Latham* (1969), describing the septo-premaxillary ligament, asserted that it represents the morphological element which transmits the septal growth thrust to the maxilla.

As regards the neurocranium, *Persson* et al. (1979) showed that fixation of the coronal suture in 9-day-old rabbits modifies the angle of the cranial vault, and that the subsequent craniectomy, performed on the 30th day, induces a complete recovery of the normal cranial morphology by the 90th day. It has been shown that early experimental closure of one neurocranial suture affects in an age-dependent manner the growth of sutures of the splanchnocranium as well (*Babler* and *Persing* 1981, 1982, 1984; *Persing* et al., 1986, 1991a; *Resnick* et al., 1990; *Wong* et al., 1991). Such interaction among the sutures was also observed by *Lin* et al. (1991) and *Marschall* et al. (1991), using animals of different species under different experimental conditions.

Recently, *Persing* et al. (1991a), studying the influence of the anterior cranial base on the mid-facial skeleton, demonstrated that expansion of the cranial base is followed by a movement of the homolateral coronal suture; the mid-facial skeleton, however, remained unchanged. The same authors (1991b) demonstrated that pathological growth of the coronal and frontonasal sutures in the rabbit leads to abnormalities similar to *Crouzon* and *Apert* syndromes.

A fundamental work on suture morphogenesis was performed by *Pritchard* et al. in 1956; they stated that the normal sutural ligament is not simple, five layers intervening between the bone edges. Each bone edge is covered by an inner cellular layer (cambial layer) and an outer fibrous layer (capsular layer) which, at the margins of the sutural surfaces, are continuous with the exocranial and endocranial periostea; between these two layers is interposed a middle layer, containing a loose vascular connective tissue. During the

Fig. 4

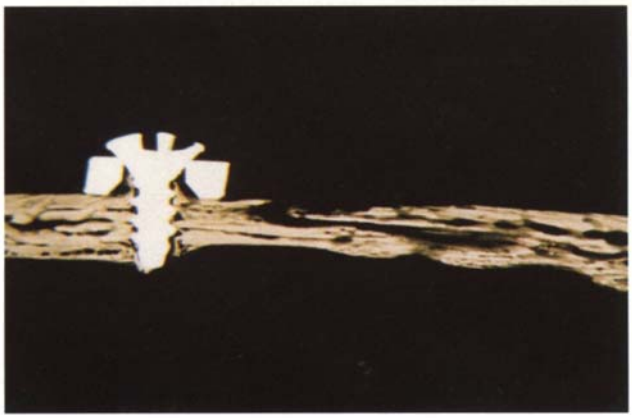

Fig. 5

Fig. 6

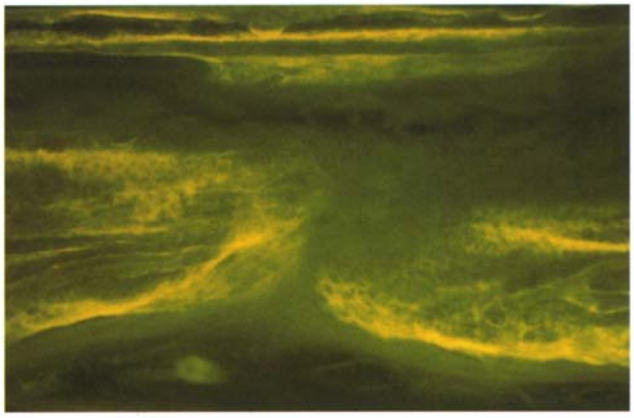

Fig. 7

Fig. 4 – Microradiograph of a parasagittal section of the unconstrained suture showing one reference screw. Field width 10 mm.
Fig. 5 – Microradiograph of a parasagittal section of the plated suture showing a screw fixing the plate. Field width 10 mm.
Fig. 6 – Micrograph under fluorescence microscopy of a parasagittal section of a non-fixed suture. Note the newly formed yellow bone labelled by the tetracycline technique. Field width 1 mm.
Fig. 7 – Micrograph under fluorescence microscopy of a parasagittal section of the plated suture. Note the uneven distribution of the fluorescent material. Field width 1 mm.

growing period, the proliferation of the middle layer, prevents synostosis. The sutures differ morphogenetically in the vault and face, though both are derived from membranous bones. In the face, the cambial and capsular layers are already present before the suture is formed, while the middle and uniting layers are derived from the mesenchyme between the approaching bone territories. In the cranium, the capsular layers are not formed until the cambial layers have almost met, while the uniting and middle zones are derived from the delamination of the fibrous ectomeninx between the bones (*Pritchard* et al., 1956).

The results of the above mentioned studies underline once again that the sutural zone not only acts as an articular structure but also as a growing centre of ossification (*Latham*, 1968; *Pritchard* et al., 1956). It is interesting to note that in our experiment, the IRF prevents the proliferation of the middle layer of the sutural membrane but not the osteogenic process in the cambial layers, as shown by the tetracycline labelling technique and by the synostosis observed 40 days after the operation. Thus, as already pointed by *Persson* et al. (1979), the suture maintains its osteogenic potential, even if fixed.

CONCLUSIONS

In maxillofacial surgery, rigid osteosynthesis devices may improve the results of surgery in comparison with wire osteosynthesis procedures. Indeed IRF has several advantages such as primary bone healing, maintaining osteotomy gaps and eliminating the need for intermaxillary fixation after surgery. However, as underlined by our findings, the incorrect use of IRF might induce serious alterations in bone growth. In fact, the present study demonstrates that IRF not only alters the macro- and microscopic morphology of the suture subjected to constraint, but also that fixed sutures behave as they do at the end of somatic growth, when the sutural membranes stop proliferating and osteogenesis continues to proceed until bone fusion. This fact strongly suggests that craniofacial growth must be monitored when IRF is

employed in children in order to avoid a delayed removal of the plate which could irreversibly stop the growth of the constrained suture.

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